Amendments to the Claims

1. **(Currently Amended)** A computer-implemented method for counting nucleic acid probe signals in a region of interest in a biological specimen, the method comprising:

obtaining, by use of a confocal microscope, a plurality of successive two-dimensional image slices of the region of interest taken at different depths along a z-axis via the confocal microscope, wherein the successive two-dimensional image slices represent respective optical sections of the region of interest at different depths of the biological specimen;

with the plurality of successive two-dimensional image slices of the region of interest, distinguishing spatially overlapping nucleic acid probe signals in the biological specimen;

in a computer system, automatically counting a number of test signals from a test probe;

in the computer system, automatically counting a number of reference signals from a reference probe; and

in the computer system, determining a ratio of the automatically-counted test signals from the test probe to the automatically-counted reference signals from the reference probe, wherein the region of interest comprises multiple cells**;**

wherein the automatically-counted test signals from the test probe comprise at least two distinguished spatially overlapping nucleic acid probe signals in the biological specimen.

2. (Original) The method of claim 1, wherein the reference probe is a polynucleotide that hybridizes to a centromere, and the number of reference signals from the reference probe approximates a nucleus count in the biological specimen.

3. (Original) The method of claim 1, wherein the reference probe recognizes a target on a same chromosome as the test probe.

4. (Original) The method of claim 1, wherein the test probe is a polynucleotide that hybridizes to a target sequence in a gene, and the reference probe is a polynucleotide that hybridizes to a reference sequence.

5. (**Currently Amended**) The method of claim ~~3~~ 4, wherein the reference probe recognizes a centromere of the same chromosome on which the gene of interest is contained.

6-8. (Canceled)

9. (Previously Presented) The method of claim 1, wherein the successive two-dimensional image slices are transformed into digital representations in which contiguous signal segments in successive optical sections are combined into a single signal in a particular optical section in which a strongest signal segment is located.

10. (Previously Presented) The method of claim 1, wherein different successive two-dimensional image slices are obtained for the test probe signals and the reference probe signals, and a quantity of test probe signals and reference probe signals are determined.

11. (Previously Presented) The method of claim 1, wherein successive two-dimensional image slices are obtained which show distinguishable test probe signals and reference probe signals, and a quantity of the test probe signals and reference probe signals are determined.

12. (Canceled)

13. (Original) The method of claim 1, wherein the ratio of signals is determined without reference to boundaries of a cell nucleus.

14. (Original) The method of claim 1, wherein the ratio of signals is determined without reference to the boundaries of a cell.

15. (Withdrawn) The method of claim 1, wherein the probe signals are visible signals from probes used with in situ hybridization of a biological sample, the method further comprising:

obtaining a plurality of images at different levels of the biological sample; and

constructing a three-dimensional image indicating discrete signals at different levels of the three-dimensional image;

wherein automatically counting comprises counting computer-identified discrete signals out of the discrete signals at different levels of the three-dimensional image.

16. (Withdrawn) The method of claim 15, wherein the three-dimensional image is constructed by determining a location of a signal segment in the different levels of the biological sample, combining overlapping signal segments in contiguous levels into a single spot signal, and separating signal segments in non-contiguous levels into different spots.

17. (Withdrawn) The method of claim 16, wherein the location of signal segments is determined by the presence of an increase in brightness intensity that indicates an increase of signal as compared to a background signal.

18. (Withdrawn) The method of claim 17, wherein the probes display fluorescent signals, and the increase in brightness intensity is associated with an increase in fluorescence compared to the background signal.

19. (Withdrawn) The method of claim 15, wherein the signals comprise test signals from a test probe and reference signals from a reference probe.

20. (Withdrawn) The method of claim 19, wherein the test probe recognizes a gene of interest, and the reference probe recognizes a chromosomal locus having an expected quantity in the biological specimen.

21. (Withdrawn) The method of claim 20, further comprising determining a ratio between the test signals and the reference signals.

22. (Withdrawn) The method of claim 21, further comprising determining:

(a) whether there is an increase in an expected ratio between the test signal and the reference signal, indicating an amplification of the gene of interest; or

(b) whether there is a decrease in the expected ratio between the test signal and the reference signal, indicating relative loss of the gene of interest.

23. (Withdrawn) The method of claim 19, wherein the test probe is selected from the group consisting of probes that recognize genes implicated or suspected in the development or progression of a tumor.

24. (Withdrawn) The method of claim 15, wherein the biological sample is in a microarray.

25. (Withdrawn) The method of claim 24, wherein the microarray comprises a tissue microarray.

26. (Withdrawn) The method of claim 25, wherein the tissue microarray comprises tissue samples of a same tissue type taken from a plurality of donor specimens.

27. (Withdrawn) The method of claim 15, wherein the plurality of images consists of between eight and thirty two images at different levels of the biological sample.

28. (Withdrawn) The method of claim 15, further comprising:

avoiding counting discrete signals having intensities exceeding a threshold intensity.

29. (Withdrawn) The method of claim 15, further comprising:

avoiding counting discrete signals having a combined intensity and area exceeding a threshold value.

30. (Withdrawn) The method of claim 15, further comprising:

avoiding counting discrete signals related to autofluorescent material.

31. (Withdrawn) The method of claim 15, further comprising:

depicting a two-dimensional image representing the three-dimensional image for consideration by a user.

32. (Withdrawn) The method of claim 31, further comprising:

emphasizing discrete signals related to autofluorescent material in the two-dimensional image.

33. (Withdrawn) The method of claim 15, further comprising:

identifying a set of one or more discrete signals as a cluster; and

counting the cluster as a number of discrete signals greater than the number of discrete signals in the set.

34. (Withdrawn) The method of claim 33, wherein the cluster is counted as a number of discrete signals indicated by applying a mapping to the number of discrete signals in the set.

35. (Withdrawn) The method of claim 33, wherein the cluster is counted as a number of discrete signals indicated by a function calibrated via manual counting of spots in a plurality of images.

36. (Withdrawn) The method of claim 33 wherein the cluster is counted as a number of discrete signals indicated by a gain factor applied to the number of discrete signals in the set.

37. (Withdrawn) The method of claim 15, wherein the plurality of images are a set of images taken during a first analysis of a first color channel, and a second set of images are taken of the biological sample for a second color channel, the method further comprising:

avoiding counting discrete signals appearing at a same location in the set of images for the first color channel and the set of images in the second color channel.

38. (Withdrawn) The method of claim 15, wherein the plurality of images are a set of images taken for a test probe, and a second set of images are taken of the biological sample for a reference probe, the method further comprising:

avoiding counting discrete signals appearing at a same location in the set of images for the test probe and the set of images for the reference probe.

39. (Withdrawn) The method of claim 15, further comprising:

receiving a directive from a user indicating counting is to be avoided for a specified portion of the biological sample; and

responsive to the directive, avoiding counting discrete signals for the specified portion of the biological sample.

40. (Withdrawn) The method of claim 15, further comprising:

receiving a directive from a user indicating counting is to be performed separately for a specified portion of the biological sample; and

responsive to the directive, separately counting discrete signals for the specified portion of the biological sample.

41-63. (canceled)

64. (**Currently Amended**) An automated system for counting nucleic acid probe signals in a region of interest in a biological specimen, the system comprising:

means for obtaining, by use of a confocal microscope, a plurality of successive two-dimensional image slices of the region of interest taken at different depths along a z-axis via the confocal microscope, wherein the successive two-dimensional image slices represent respective optical sections of the region of interest at different depths of the biological specimen;

means for counting a number of test signals from a test probe;

means for counting a number of reference signals from a reference probe;

means for distinguishing spatially overlapping nucleic acid probe signals in the biological specimen via the plurality of successive two-dimensional image slices of the region of interest; and

means for determining a ratio of the counted test signals from the test probe to the counted reference signals from the reference probe, wherein the region of interest comprises multiple cells**;**

wherein the counted test signals from the test probe comprise at least two distinguished spatially overlapping nucleic acid probe signals in the biological specimen.

65. (Canceled)

66. (Canceled)

67. (Previously Presented) One or more computer-readable media comprising computer-executable instructions for performing the method of claim 9.

68. (**Currently Amended**) One or more computer-readable media having computer-executable instructions for performing a method for counting nucleic acid probe signals in a region of interest in a biological specimen, the method comprising:

obtaining via confocal microscopy a plurality of successive two-dimensional image slices of the region of interest taken at different depths along a z-axis via the confocal microscopy, wherein the successive two-dimensional image slices represent respective optical sections of the region of interest at different depths of the biological specimen;

with the plurality of successive two-dimensional image slices of the region of interest, distinguishing spatially overlapping nucleic acid probe signals in the biological specimen;

automatically counting a number of test signals from a test probe;

automatically counting a number of reference signals from a reference probe; and

determining a ratio of the automatically-counted test signals from the test probe to the automatically-counted reference signals from the reference probe, wherein the region of interest comprises multiple cells**;**

wherein the automatically-counted test signals from the test probe comprise at least two distinguished spatially overlapping nucleic acid probe signals in the biological specimen.